Rapid and Efficient Conversion of Integration-Free Human Induced Pluripotent Stem Cells to GMP-Grade Culture Conditions.

Journal: PLoS One

Publication Year: 2014

Authors: Jens Durruthy-Durruthy, Sharon F Briggs, Jason Awe, Cyril Y Ramathal, Saravanan Karumbayaram, Patrick C Lee, Julia D Heidmann, Amander Clark, Ioannis Karakikes, Kyle M Loh, Joseph C Wu, Andrew R Hoffman, James Byrne, Renee A Reijo Pera, Vittorio Sebastiano

PubMed link: 24718618

Funding Grants: Autologous iPSC Therapy for Urinary Incontinence

Public Summary:
Recent advancement in the field of iPSC (induced pluripotent stem cell) technology suggests that iPSCs can be a potent source of autologous cells for patient-specific cell based therapies. However the current exogenous DNA-free based methods available for reprogramming of human fibroblasts involve 1) possibility of integration of very small fragments of foreign DNA (e.g: Episomal vectors and minicircles) or 2) in-efficient techniques (protein-based or Sendai virus) or 3) expensive method of using mRNAs. Also, to make this technology user friendly it is critical to have the derivation process to be rapid, efficient, and cost effective and manufactured through Good Manufacturing Practice (GMP). Additionally, rigorous tests should also be performed to exclude the presence of adventitious agents that could have been present in the research-grade reagents used during the cell derivation process. Modified synthetic mRNAs have been shown to reprogram fibroblasts to a pluripotent state making them safer for potential clinical application. Here, we developed and optimized, fully chemically defined and feeder-free protocol for the derivation of hiPSCs using synthetic mRNAs. This protocol is highly reproducible across multiple lines and can generate human iPSCs efficiently and rapidly and completely free of any xenogenic factors. Hence this method can be easily transferred to GMP compliant conditions for making clinical grade cells that would be safe for human consumption. In the present study, we were able to derive hiPSC lines from adult dermal fibroblasts in less than two weeks. The lines were also successfully tested for their identity, purity, stability and safety at a GMP facility and were cryopreserved for potential clinical use. To our knowledge, these are the first integration-free iPSCs lines that were reproducibly generated through synthetic mRNA reprogramming that could be putatively used for clinical purposes.

Scientific Abstract:
Data suggest that clinical applications of human induced pluripotent stem cells (hiPSCs) will be realized. Nonetheless, clinical applications will require hiPSCs that are free of exogenous DNA and that can be manufactured through Good Manufacturing Practice (GMP). Optimally, derivation of hiPSCs should be rapid and efficient in order to minimize manipulations, reduce potential for accumulation of mutations and minimize financial costs. Previous studies reported the use of modified synthetic mRNAs to reprogram fibroblasts to a pluripotent state. Here, we provide an optimized, fully chemically defined and feeder-free protocol for the derivation of hiPSCs using synthetic mRNAs. The protocol results in derivation of fully reprogrammed hiPSC lines from adult dermal fibroblasts in less than two weeks. The hiPSC lines were successfully tested for their identity, purity, stability and safety at a GMP facility and cryopreserved. To our knowledge, as a proof of principle, these are the first integration-free iPSCs lines that were reproducibly generated through synthetic mRNA reprogramming that could be putatively used for clinical purposes.